

THE FIRST AND SECOND LARVAL INSTARS AND THE EGG  
OF *PARASIMULIUM STONEI* PETERSON  
(DIPTERA: SIMULIIDAE)

A. BORKENT AND D. M. WOOD

Biosystematics Research Institute, Agriculture Canada, Ottawa, Ontario  
K1A 0C6, Canada.

---

*Abstract.* — The first instar larva of *Parasimulium stonei* has only a single minute labral fan ray and apparently lacks an anteromedian palatal brush. The sensilla of the maxillary palpus are arranged laterally on its apical portion and spicules are also present. Second instar larvae have a labral fan similar in form to that of most Prosimuliini. Some of these data suggest a relationship with typical members of the Prosimuliini, thus contradicting previous suggestions, based on study of adults, of a sister group relationship with all other Simuliidae. The shape of the egg is essentially the same as that of other blackflies, triangular, with a dorsal, transverse bulge.

---

Rediscovery of adults of *Parasimulium crosskeyi* Peterson, nearly 50 years after the last known specimens of the genus were collected, has led to renewed interest in the genus and its phylogenetic position (Wood and Borkent, 1982) and intensified the search for immature stages. Smart (1945) erected the subfamily Parasimuliinae for this genus alone, and several subsequent authors (Crosskey, 1969; Rubtsov, 1974; Peterson, 1977) followed this arrangement. These authors put the Parasimuliinae at the beginning of their classifications, with the statement or implication that it represented the most primitive member of the family. The notion that *Parasimulium* is the most primitive simuliid was supported by Wood and Borkent (1982) who, in examining adults, postulated a sister-group relationship between *Parasimulium* and all other blackflies. Information about the immature stages would test this idea and hopefully add significantly to our understanding of the polarity of character states within Simuliidae. In this paper we describe and discuss the newly discovered first and second instar larvae of *Parasimulium stonei* Peterson and reassess our original hypothesis.

In spite of intensive searching in a variety of microhabitats in the area of Wahkeena Creek, Benson State Park, Oregon, where the adults of *P. crosskeyi* Peterson were found to be common, we were unable to locate immature stages. In May 1983 one of us (DMW) discovered male and female adults of *Parasimulium stonei* beside a small rivulet near Humboldt Redwoods State Park, Humboldt County, California and was able to obtain a few eggs from a female of this species. This female was simply placed in a transparent, plastic shell vial whose open end was closed with a piece of maple leaf. Eggs were laid in the moisture that formed on the side of the vial. These small transparent eggs were then flooded

with distilled water and placed in a refrigerator. Some of them hatched sometime during the following winter (while under refrigeration), but because of their minute size and transparency, the larvae were not discovered until after they had all died. Some of the first instar larvae had molted to the second instar. They were then preserved in 70% ethanol. These specimens formed the basis of the following description and analysis.

In this paper we follow the general morphological terms given by Teskey (1981). For specific structures, we follow those given by Craig (1974) for the labrum, by Craig and Borkent (1980) for the maxillary palpus and by Craig and Batz (1982) for the antenna and associated sensilla.

#### DESCRIPTION

**Egg.**—When first laid, the eggs of *P. stonei* were transparent but by the time of hatching had acquired a light brown pigmentation. The shape of the egg was similar to that of other blackflies, with a swollen dorsal transverse bulge (Davis, 1971) and we are not able to see any essential difference between it and the eggs of other blackflies with which we are familiar (those of species of *Gymnopaia* Stone, *Twinnia* Stone and Jamnback, *Prosimulium* Roubaud, *Simulium* Latreille). This egg shape is evidently a synapomorphic character state of the Simuliidae since the eggs of other Nematocera are either oval or nearly cylindrical in outline.

Eggs of *P. stonei* were 243–265  $\mu\text{m}$  ( $n = 3$ ) in length. Some of the eggs we observed held mature first instar larvae and in such instances the only part of the larva which could be seen was the dark egg burster on the dorsum of the head capsule.

**First instar larva.**—General body form typically simuliid like, with details of anterior proleg and posterior anal hooks not discernable; head capsule transparent except for black egg burster (Fig. 1a); eye spot lacking; antenna (Fig. 1c) a single article with terminal uniporous cone sensillum, two basal multiporous peg sensilla (mps) and trichoid sensillum 2 (ts2), associated bacteria-covered multiporous sensillum (bms) (no bacteria present in our specimens) (Fig. 1b) and trichoid sensillum 1 (ts1); labrum (Fig. 2a, b) with setae 5C, 6C mesal to antenna (Fig. 1b), 2C, 4C present, 3C questionably present, single labral fan ray situated laterally, about half way between setae 2C and 4C (Fig. 1d, e); anterior palatal brush apparently absent; maxillary palpus with complete complement of sensilla (Fig. 2c, d) (Craig and Borkent, 1980: Fig. 2) but 1E, 2E and G not discernable (probably too dirty), sensilla arranged laterally on apical portion of palpus and in circular configuration, spicules present (Fig. 1b); hypostoma (Fig. 2e, f) with prominent triangular median tooth, shorter lateral tooth and scarcely discernable intermediate teeth.

**Second instar larva.**—Similar to first instar in general body form; head capsule lacking pigmentation, except for a tiny eye spot and sclerotized hypostoma; antenna similar to first instar but basal article supporting two multiporous sensilla, more elongate; labral fan (Fig. 3a) well developed, with single, anterior, medially directed, fringed ray, behind which is a short series of rays, also directed medially, primary fan with over 10 rays, secondary triangular fan with five rays, restricted to lateral margin; mandible with apical spicules and teeth (Fig. 3d); maxillary palpus sensilla not readily interpretable (too dirty) but all arranged apically on palpus, spicules absent; hypostoma (Fig. 3e) with median tooth long, sharply

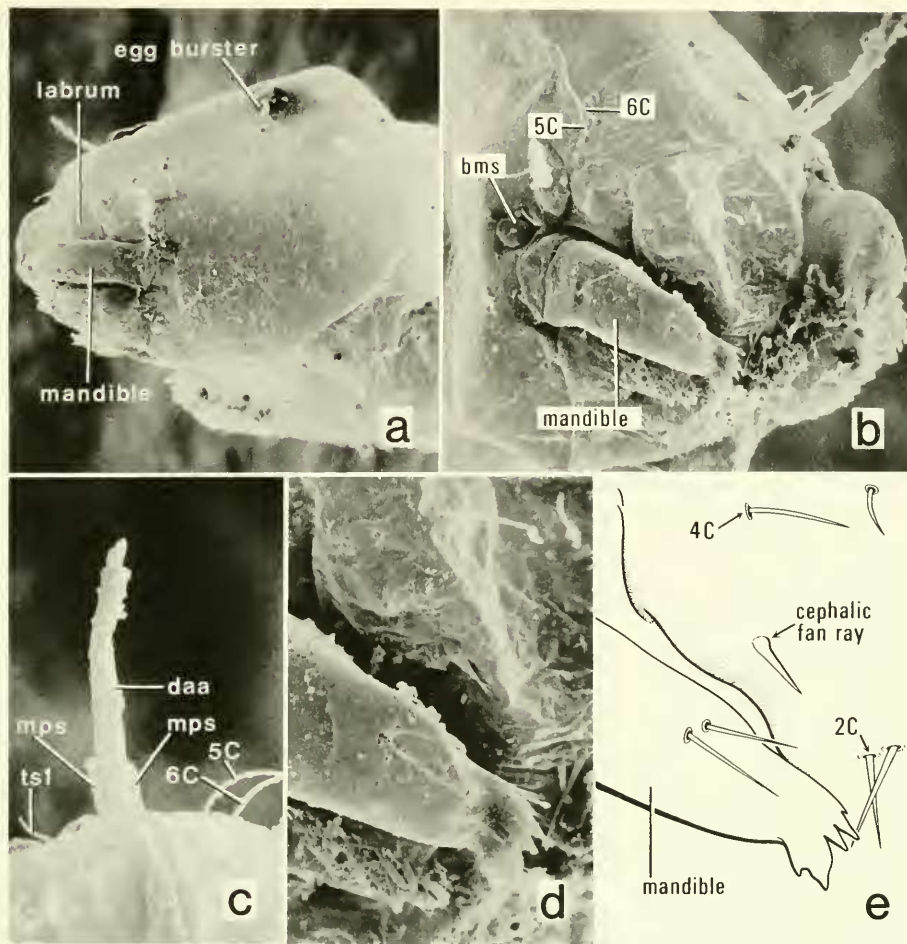


Fig. 1. First instar of *Parasimulium stonei*. a, Dorsolateral view of head capsule. b, Anterolateral view of head capsule, *bms* (bacteria-covered multiporous sensillum). c, Anterior view of right antenna, *mps* (multiporous peg sensillum), *daa* (distal antennal article), *ts1* (trichoid sensillum). d, Ventral view of right mandible and labrum. e, Drawing of figure d.

pointed, as long as broad, submedian tooth vestigial; all remaining teeth (lateral and intermediate) arising in two groups at apex of long, parallel-sided, stalk-like base.

#### DISCUSSION

A few of the characters described here can be interpreted from either a phylogenetic or an ecological perspective.

Courtney (1986) has discovered the larvae and pupae of *P. crosskeyi* Peterson in the hyporheic zone of Wahkeena Creek, Oregon. We assume that the immatures of *P. stonei* will be discovered in a similar microhabitat. Their presence in these subsurface creek gravels would correlate with the lack of eyespots in the larvae of *P. stonei* described above.

Some authors have argued that the virtual absence (i.e. reduction to a few

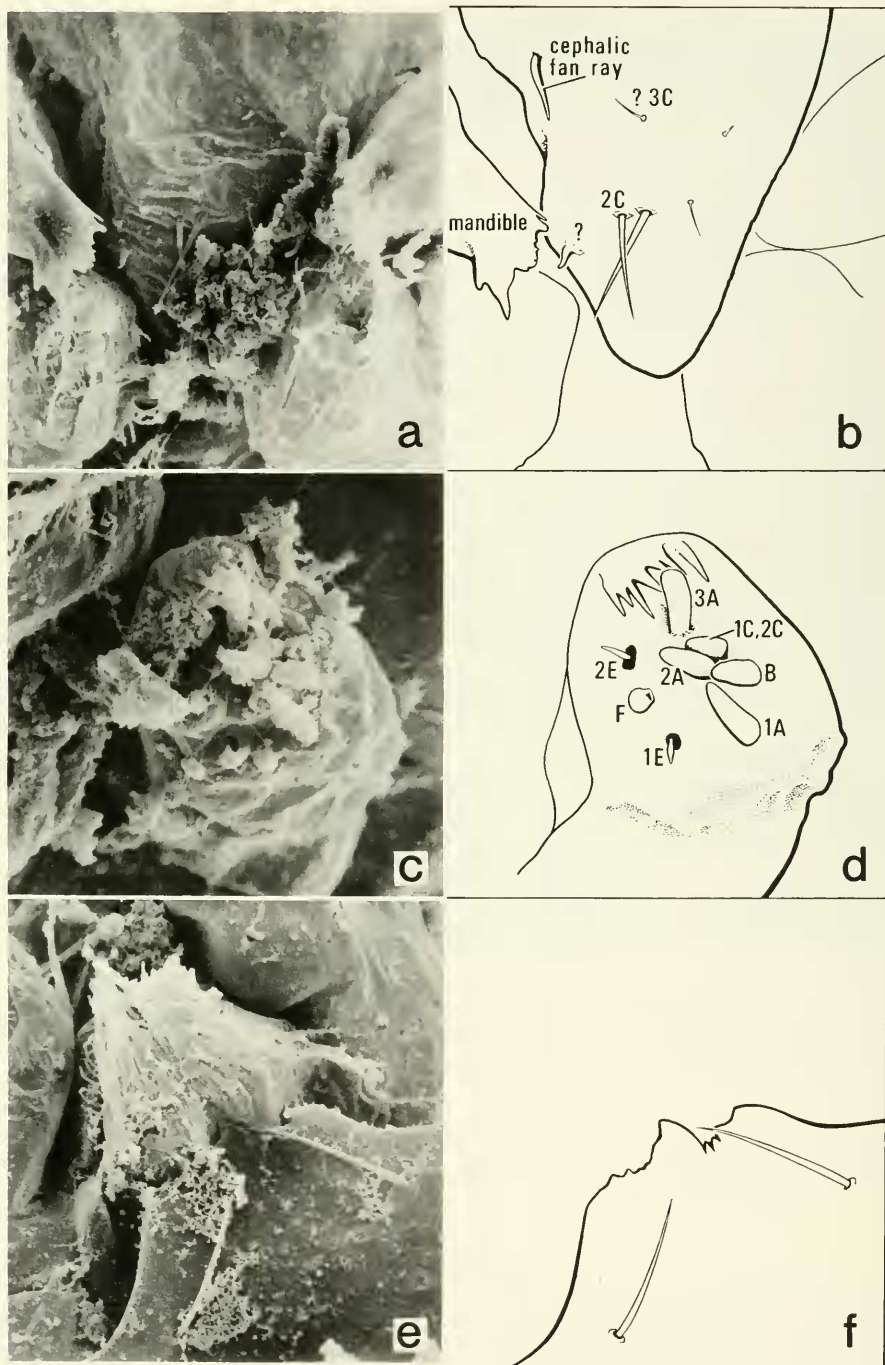


Fig. 2. First instar of *Parasimulium stonei*. a, Anterior view of labrum. b, Drawing of labrum in a. c, Ventral view of maxillary palpus. d, Drawing of palpus in c. e, Ventral view of hypostoma. f, Drawing of hypostoma in e.



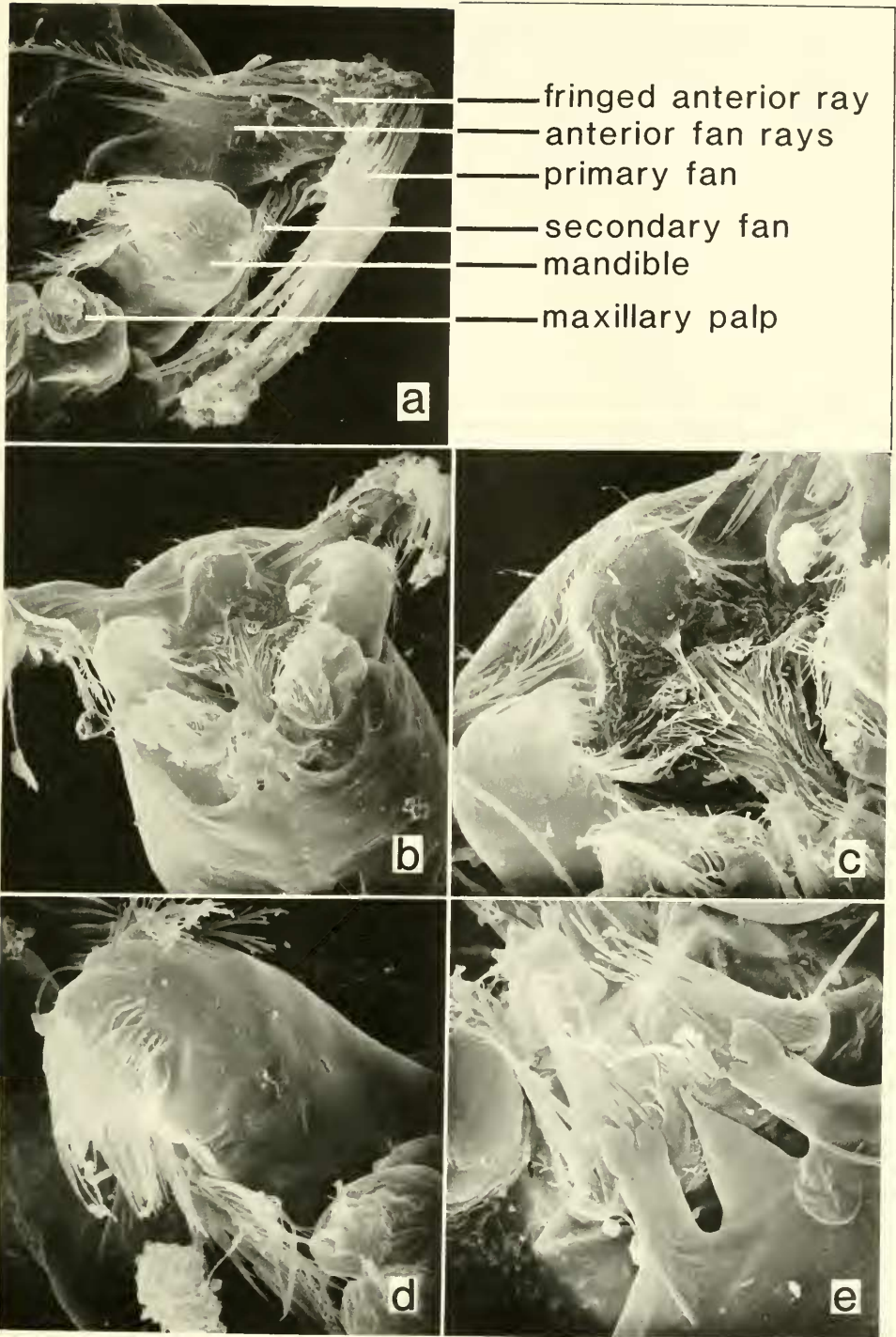


Fig. 3. Second instar of *Parasimulium stonei*. a, Ventral view of left labral fan. b, Anteroventral view of mouthparts. c, Ventral view of labrum. d, Ventral view of left mandible. e, Ventral view of hypostoma.

minute fan rays) of labral fans in first instar *Prosimulium*, and in all instars of *Gymnopais* and *Twinnia*, is the plesiomorphic condition in the Simuliidae (Davies, 1960, 1965; Rubtzov, 1974). Craig (1974) and Wood (1978), however, have argued that the lack of fans must be considered apomorphic. Cladistically closely related families (Culicidae, Dixidae, Ptychopteridae) possess well developed homologous labral fans in each instar. Such comparisons strongly suggest that greatly reduced labral fans of some first instar larval simuliids must be considered apomorphic. First instars of *Parasimulium* share this character state with some other members of the Prosimuliini, viz, *Prosimulium*, *Gymnopais*, *Twinnia*, and *Crozetia* (Craig, 1974), suggesting that all these genera form a monophyletic group within Prosimuliini (Figs. 4A, 5). Not all members of Prosimuliini (sensu Crosskey) lack labral fans in the first instar; those of at least one species of *Gigantodax* and one of the Australian species assigned to *Cnephia*, possess fans of normal development for first instars, showing that the Prosimuliini cannot be defined on the basis of this character state. A more detailed understanding of the distribution of this character state would be of fundamental interest in the phylogenetic interpretation of simuliids.

First instars of *Prosimulium*, *Gymnopais*, and *Twinnia* also have paired series of scoop-shaped, apically fringed, anteromedian palatal brushes (Fig. 4A, from Craig, 1974). Such modifications of the anteromedian palatal brushes in the first instar are not known in any outgroup, and probably represent a synapomorphy of the three genera. Unfortunately, many genera remain to be surveyed for this character state. These brushes appeared to be absent in our specimen of *Parasimulium*. If they are present but not visible in our material, they are not as closely associated with seta 2C as they are in *Twinnia* (Craig, 1974: fig. 25). It will be important to determine, with the help of better specimens, whether these brushes are as highly modified in *Parasimulium* as they are in the other three genera; if they are it would be another synapomorphy linking *Parasimulium* to this group of genera.

The secondary labral fan, present in almost all simuliids that have labral fans (it is apparently absent in *Crozetia*), is shorter and smaller than the primary fan, and arises ventral to it (Figs. 3a, 4B). Rays forming the secondary fan are merely a continuation of the circle of rays that form the primary fan, separated from the last ventrolateral primary ray by a short section of aborted rays that resemble lamellae. The most proximal ray forms the anterior, or leading, edge of the secondary fan, while the most distal of the secondary rays forms the posterior edge. Our studies of the shape and arrangement of the fan rays of the secondary labral fan of various simuliids have shown that there are two discrete types of secondary fan, differing in the number and curvature of the rays (Wood et al., 1963). Crosskey (1969) pointed out that some members of his tribe Prosimuliini had a subtriangular flattened secondary fan, in which the rays were rather broad and blade-like (Fig. 4B, *Prosimulium*), and in which the apices of the fan formed a rather straight line parallel to the fan stalk. The secondary fan thus closes the space between the base of the stalk and the primary fan. In contrast, in all members of his Simuliini the secondary fan is cup-shaped and consists of finer, much more numerous, curved rays, the whole fitting within the contours of the primary fan (Fig. 4B, *Simulium*).

We have found the first type of secondary fan in the second instar of *Parasi-*

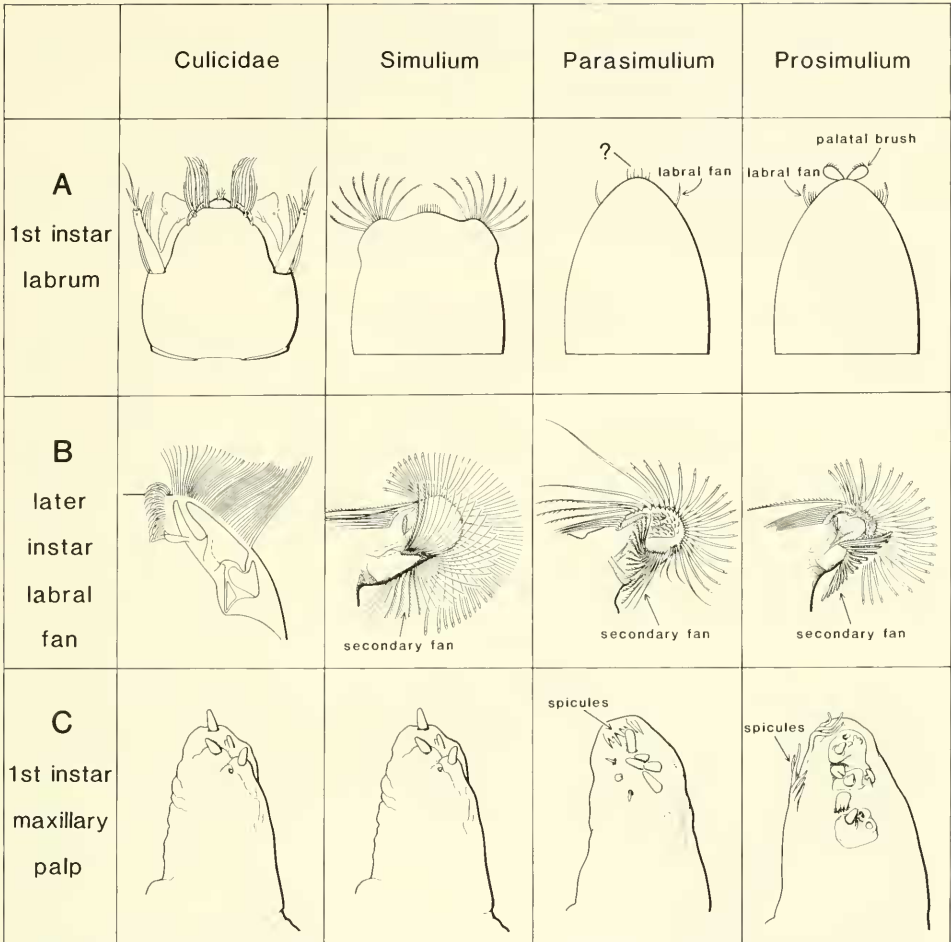


Fig. 4. Schematic representation of transformation series of character states within the Simuliidae and outgroup (as represented by Culicidae). Transformation A is partially taken from Craig, 1974.

*mulium stonei*; although containing more rays than that of the species of *Prosimulium* we examined, the shape of the fan is flattened and triangular, and the line formed by the apices of the fan is straight and oriented parallel to the fan stalk. We have examined various species of other genera and found the same type of arrangement. These were: several Australian species still assigned to *Cnephia* (but undoubtedly belonging elsewhere), viz., *aurantiacum* Tonnoir, *fuscoflava* Mackerras and Mackerras, *orientalis* Mackerras and Mackerras, *strenua* Mackerras and Mackerras, and *tonnoiri* Drummond; several neotropical species, *Cnesia dissimilis* (Edwards), *Araucnephia montana* (Phillipi) and several species of *Gigantodax* Enderlein; and the Afrotropical *Prosimulium morotoense* McCrae and Prentice. The condition in the remaining Australian species of "*Cnephia*," of Afrotropical *Prosimulium*, and of the other neotropical Prosimuliini remains to be determined. The secondary fan of *Ectemnia taeniatifrons* Enderlein also appeared to be of this type but was folded up in all our specimens.



The second type, with a cup-shaped secondary fan, was found in all members of *Simulium* and *Austrosimulium* examined, which Crosskey placed in his Simuliini, but was also found in some of his Prosimuliini, e.g. *Cnephia sensu stricto*, *Metacnephia*, and *Stegopterna*.

Wood (in prep) suggests that the cup-shaped second type of secondary fan is a derived condition within the Simuliidae. The presence of a subtriangular secondary fan in *Parasimulium* and some Prosimuliini must therefore be considered a symplesiomorphy and cannot be used to elucidate the phylogenetic position of the genus.

Craig and Borkent (1980) provided a comparative morphological analysis of the maxillary palpus of larval blackflies and some other nematocerous Diptera. The results showed that in nearly all taxa examined the sensilla were in a circular arrangement at the apex of the palpus. The first instars of the species of *Prosimulium*, *Gymnopais* and *Twinnia* studied were unique in having the sensilla positioned, first of all, laterally on the palpus and secondly, in a linear arrangement. The palpal sensilla of *Parasimulium stonoi* are arranged laterally on the palpus but retain the plesiomorphic condition of being more or less in a circular configuration (Fig. 4C). These data also suggest that *Parasimulium* is the sister group to *Prosimulium* plus *Gymnopais* plus *Twinnia*.

Craig and Borkent (1980) also noted that there were sclerotized plates surrounding the apical sensilla of the maxillary palp of later instars of *Prosimulium*, *Gymnopais* and *Twinnia*. Among these plates were spicules that were unique to these three genera and which were therefore considered to be a synapomorphy. Of the first instar larvae of these three genera, only *Prosimulium* and *Gymnopais* had the spicules and they were lacking in those of *Twinnia*. First instar *Parasimulium stonoi* exhibit the spicules, but these appear to be lacking in the second instar. In our scheme here, we suggest that the spicules are a synapomorphy of all four genera but that they have been secondarily lost in the first instar of *Twinnia*. The character state transformation is summarized in Fig. 4C.

Based on their study of adult structures, Wood and Borkent (1982) provided three character states which indicated the monophyly of Simuliidae, exclusive of *Parasimulium*. These were: (1) male eye divided by a line of discontinuity into large upper facets and small lower facets; (2) branches of radial sector ( $R_{2+3}$  and  $R_{4+5}$ ) closely approximated (more widely separated in *Parasimulium*); and (3) sternite 10 of female divided medially (undivided in *Parasimulium* and other Culicomorpha). The genus *Parasimulium* was therefore considered the sister group to all other blackflies.

As discussed above, information from the first instar larvae indicates that *Parasimulium* may be the sister group to a monophyletic assemblage containing *Prosimulium* plus *Gymnopais* plus *Twinnia*. Wood (1978) has analyzed the relationship between *Prosimulium*, *Gymnopais* and *Twinnia*. Our evidence further suggests that *Crozetia* is the sister group to this assemblage of four genera (Fig. 5).

We are presently unsure of how to interpret these conflicting synapomorphies between the adults and larvae, although our inclination is to put greater weight on the larval characters. We know that divided adult eyes is susceptible to homoplasy (present also in male Bibionidae and both sexes of Axymyiidae), and that wing venation may be a poor indicator of relationship, especially considering the small difference between *Parasimulium* and *Prosimulium*. Interpretation of



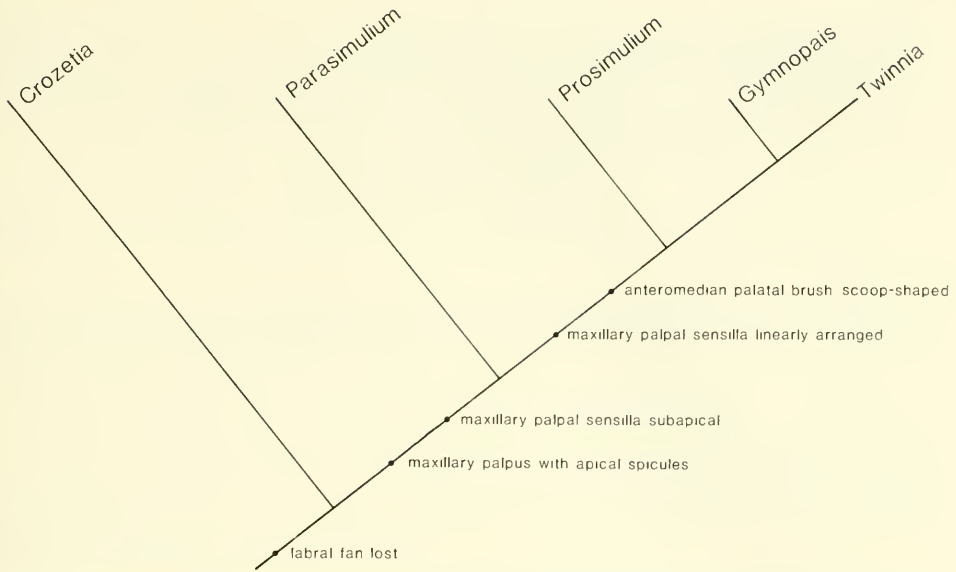


Fig. 5. Cladogram showing relationship of *Parasimulium* to some Prosimuliini. Character states are those of the first instar larva.

the incompletely divided sternite 10 of the female of *Parasimulium* remains a difficult problem as there is little doubt of the completely divided nature of the sternite in other Simuliidae contrasting with a complete sternite in the outgroup families. Although we cannot confidently resolve the present conflict, we trust that further study of other characters and their distribution will help to shed some light.

Our present analysis was possible only because of some comparative morphological studies carried out by previous workers (Craig, 1974; Craig and Borkent, 1980). While we applaud the availability of such investigations we also regret the lack of comparable studies of other structures such as the antennae, mandible and hypostoma. We hope that future work will allow the interpretation of such characters as we have described here.

#### ACKNOWLEDGMENTS

We appreciate the constructive criticism of our manuscript by our colleagues Guy Shewell and Mike Sharkey. Ralph Idema assisted with labelling of the plates and drafted Fig. 4. Barry Flahey drafted Fig. 5. We appreciate also the assistance of the staff of the Electron Microscope Unit of the Cell Biology Research Institute, Agriculture Canada, Ottawa.

#### LITERATURE CITED

- Courtney, G. W. 1986. Discovery of the immature stages of *Parasimulium crosskeyi* Peterson (Diptera: Simuliidae), with a discussion of a unique black fly habitat. Proc. Entomol. Soc. Wash. 88: 280-286.
- Craig, D. A. 1974. The labrum and cephalic fans of larval Simuliidae (Diptera: Nematocera). Can. J. Zool. 52: 133-159.

- Craig, D. A. and H. Batz. 1982. Innervation and fine structure of antennal sensilla of Simuliidae larvae (Diptera: Culicomorpha). *Can. J. Zool.* 60: 696-711.
- Craig, D. A. and A. Borkent. 1980. Intra- and inter-familial homologies of maxillary palpal sensilla of larval Simuliidae (Diptera: Culicomorpha). *Can. J. Zool.* 58: 2264-2279.
- Crosskey, R. W. 1969. A re-classification of the Simuliidae (Diptera) of Africa and its islands. *Bull. Br. Mus. (Nat. Hist.) Entomol. Suppl.* 14. 195 pp. + 1 pl.
- Davies, L. 1960. The first instar of a species of *Prosimulium* (Diptera: Simuliidae). *Can. Entomol.* 92: 81-84.
- . 1965. The structure of certain atypical Simuliidae (Diptera) in relation to evolution within the family, and the erection of a new genus for the Crozet Island blackfly. *Proc. Linn. Soc. Lond.* 176: 159-180.
- Davis, C. C. 1971. A study of hatching process in aquatic invertebrates. XXV. Hatching in the blackfly, *Simulium* (probably *venustum*) (Diptera, Simuliidae). *Can. J. Zool.* 49: 333-336.
- Peterson, B. V. 1977. A synopsis of the genus *Parasimulium* Malloch (Diptera: Simuliidae), with descriptions of one new subgenus and two new species. *Proc. Entomol. Soc. Wash.* 79: 96-106.
- Rubtzov, I. A. 1974. On the evolution, phylogeny and classification of the black flies (Simuliidae, Diptera). (In Russian.) *Tr. Zool. Inst. Acad. Nauk SSSR* 53: 230-281.
- Smart, J. 1945. The classification of the Simuliidae (Diptera). *Trans. R. Entomol. Soc. Lond.* 95: 463-528.
- Teskey, H. J. 1981. Ch. 3. Morphology and terminology—larvae, pp. 65-88. *In* J. F. McAlpine et al. (eds.), *Manual of Nearctic Diptera*, Vol. 1, Research Branch, Agriculture Canada, Monograph 27. 674 pp.
- Wood, D. M. 1978. Taxonomy of the nearctic species of *Twinnia* and *Gymnopais* (Diptera: Simuliidae) and a discussion of the ancestry of the Simuliidae. *Can. Entomol.* 110: 1297-1337.
- Wood, D. M. and A. Borkent. 1982. Description of the female of *Parasimulium crosskeyi* Peterson (Diptera: Simuliidae), and a discussion of the phylogenetic position of the genus. *Mem. Entomol. Soc. Wash.* 10: 193-210.
- Wood, D. M., B. V. Peterson, D. M. Davies, and H. Gyorkos. 1963. The black flies (Diptera: Simuliidae) of Ontario. II. Larval identifications, with descriptions and illustrations. *Proc. Entomol. Soc. Ont.* 93 (1962): 99-129.